Wound healing acceleration in inflammation phase of post-tooth extraction after aerobic and anaerobic exercise

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ORIGINAL ARTICLE

Wound healing acceleration in inflammation phase of post-tooth extraction after aerobic and anaerobic exercise

Accélération de la guérison des blessures dans la phase inflammatoire d'extraction dentaire après un exercice aérobie et anaérobie

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KEYWORDS
Physical exercise;
PMN;
Macrophage;
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Wound healing

Summary
Background. — Tooth extraction leads a wound healing that is expected to be physiologically healed in a short time. Both aerobic and anaerobic physical exercises are scientifically proven to enhance various biological functions as well as to accelerate the wound healing in oral mucosa. Aerobic exercise increases tissue oxygenation, accelerates the wound healing and this is different to anaerobic exercise which is considered to produce more free radicals and interferes the wound healing process.
Purpose. — To observe the differences of wound healing effectiveness in the inflammatory phase after tooth extraction based on the number of PMN and macrophage cells in Wistar rats (Rattus norvegicus) that received aerobic and anaerobic exercise.
Methods. — Forty-two rats were divided into three groups: control group (K), aerobic exercise group (P1), and anaerobic exercise group (P2). In each group the number of PMN cells was calculated one day after tooth extraction and the counting of the macrophage cells were performed on the other groups two days later. Data was analyzed statistically using Kruskal Wallis, Levene's test, and proceed with Oneway Anova.
Results. — The highest number of PMN and macrophage cells was obtained in P1 group and the lowest results were in K group. The data between groups demonstrated significant differences.

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Conclusion. — This study represents significant differences in the number of PMN and macrophage cells between aerobic and anaerobic exercises in wound healing after tooth extraction wh can be a strong indicator of wound healing acceleration in the inflammatory phase.

MOTS CLÉS
Exercice physique ;
PMN ;
Macrophage ;
Extraction dentaire ;
Cicatrisation

Résumé
Contexte. — L'extraction de dents entraîne la cicatrisation d'une plaie qui devrait être guérie physiologiquement dans un court laps de temps. Il a été prouvé scientifiquement que les exercices physiques aérobies et anaérobies améliorent diverses fonctions biologiques et accélèrent la cicatrisation de la plaie dans la muqueuse buccale. L'exercice aérobie augmente l'oxygénation des tissus, accélère la cicatrisation de la plaie. Cet exercice est différent de l'exercice anaérobie, censé produire plus de radicaux libres et interférer avec le processus de cicatrisation de la plaie.
Objectif. — Observer les différences d'efficacité de cicatrisation des plaies dans la phase inflammatoire après l'extraction d'une dent en fonction du nombre de cellules PMN et de macrophages chez les rats Wistar (Rattus vegicus) ayant fait l'objet d'exercices aérobies et anaérobies.
Méthodes. — Quarante-deux rats ont été divisés en trois groupes : groupe témoin (K), groupe d'exercice aérobique (P1) et groupe d'exercice anaérobie (P2). Dans chaque groupe, le nombre de cellules PMN a été calculé un jour après l'extraction dentaire et le c tage des cellules macrophages a été effectué sur les autres groupes deux jours plus tard. Les données ont été analysées statistiquement à l'aide du test de Kruskal Wallis, de Levene, puis de Oneway Anova.
Résultats. — Le plus grand nombre de cellules PMN et macrophages a été obtenu dans le groupe P1 et les plus faibles résultats dans le groupe K. Les données entre les groupes ont montré des différences significatives.
Conclusion. — Cette étude représente des différences significatives dans le nombre de cellules PMN et de macrophages entre les exercices aérobies et anaérobies dans la cicatrisation des plaies après une extraction dentaire, ce qui peut être un indicateur fort de l'accélération de la cicat tion des plaies dans la phase inflammatoire.

1. Introduction

Tooth extraction is the most frequent surgical procedure in dental medicine [1]. Tooth extraction will cause sores on the socket which then physiological healing which can occur acute or chronic. Chronic wound occurs when the healing process in acute wounds is accompanied by complications that cause the delaye ound healing process and more difficult to handle. The wound healing process is a process of repairing or changing tissue and functional capacity that is damaged due to injury. It is physiologically divide to 4 overlapping sequential phases, they are hemostasis phases, the inflammatory phase, the proliferation phase, and the maturation/remodeling phase [2,3].

Inflammation phase begin with the release of Polymorphonuclear Neutrophilic (PMN)/neutrophil which plays role as phagocytosis. Neutrophils are motile cells that enter the socket after wound occurred and it works during the first 24—48 hours. After 2—3 days, the population neutrophils shift and the wound area are dominated by monocytes which differentiate into macrophages. PMN and macrophages not only phagocytes antigens but also synthesize cytokines that will initiate angiogenesis process, and fibroblast that will affect the next healing phase. Inflammation phase lasted from the onset of injury until the third day [4,5].

Physical exercise has an important meaning in maintaining health when exercise is carried out at the compatible level of intensity, duration, and frequency [6]. Generally, physical exercise influences various biological functions in the form of positive influences, which are to improve bodily functions and negative effects [7]. There are two types of physical exercises namely aerobic exercise and anaerobic exercise. Aerobic exercise is an activity that depends on the availability of oxygen to help the process of forming Adenosine Tri Phosphate (ATP) which will be used as an energy source. Aerobic exercise as an activity that uses a large group of muscles and can be carried out continuously for a long time. Anaerobic exercise is a high-intensity activity that requires energy quickly in a short time but cannot be carried out continuously for a long duration. Anaerobic physical exercise does not require oxygen in the process of forming its energy source because it depends on the energy stored in the muscle and results from anaerobic glycolysis [8,9].

The acceleration of healing in the inflammatory phase is significantly determined by the number of PMN and macrophage cells, and no previous studies differentiated the wound healing due to aerobic and anaerobic physical exercise at this phase [10]. We tried to conduct a further study related to the comparison of the quality of wound healing after dental extraction measured by the number of PMN and macrophage cells in the inflammatory phase due to aerobic and anaerobic physical exercise.

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Table 1 The result of sample observation in control group (K), aerobic exercise group (P1), and anaerobic exercise group (P2).

Sample group	Mean	Std Deviation	*P*-value Normality[a]	*P*-value Homogeneity[b]	Anova
PMN cell				0.001	0.001[c]
(K) Control	22.2286	7.18928	0.200		
P1 (aerobic)	44.6000	5.46748	0.200		
P2 (anaerobic)	35.2000	1.30639	0.200		
Macrophage cell				0.021	0.000[c]
Control	16.8571	3.52839	0.200		
P1 (aerobic)	42.5714	7.52190	0.136		
P2 (anaerobic)	29.6571	1.75010	0.200		

[a] Data distributed normally if *P*-value > 0.05.
[b] Homogeny if *P*-value > 0.05.
[c] Significant if *P*-value < 0.05.

2. Materials and methods

This research is a laboratory experimental study with the post-test only control group design. It was conducted in accordance to ethical clearance certificate number 232/HRECC.FODM/X/2018, which was declared by ethical committee of the Faculty of Dental Medicine Universitas Airlangga.

The animals were divided into 3 groups, consisted of control group (K) that was not given any treatment, group 2 (aerobic group), and group 3 (anaerobic group). The rats in P1 group were given aerobic exercise with load 3% of its body weight. Exercises carried out for 20 minutes with 1-minute rest are given at every 5 minutes of the exercise interval for 14 days. Rats in P2 group were given anaerobic exercise with high intensity as much as 10% of body weight on first and second day, 13% on third and fourth day, and 15% on fifth and sixth day respectively, and repeated up to 14 days. The anaerobic exercise was performed for 80 seconds with 10 seconds of rest given at every 10 seconds of training interval. The applied load was in the form of a paper clip tied with a thread placed on 1/3 of the base of the tail of the animal [11,12].

On day 15, all of the rats' tooth were extracted and tissue around the tooth extraction were taken on the first day after tooth extraction to observe the PMN cells amount, and on the third day after tooth extraction, tissue were taken to observe macrophage cells using HE stained and seen on light microscope with 400 × magnification [13].

The data obtained from the study were tested for normality first using the Kolmogorov-smirnov test and tested its homogeneity with Leven's test. Research data were normally distributed ($P > 0.05$) followed by one-way Anova analysis [14].

3. Results

The highest mean PMN and macrophages were in the aerobic treatment group and the lowest were in the control groups. Through Kolmogorov-Smirnov test, PMN and macrophages in all groups are normally distributed ($P > 0.05$) meanwhile in the homogeneity test used Levene's test and the results obtained PMN and macrophages ($P < 0.05$) it can be concluded that the data was not homogenous. Followed by a whole different test group on PMN and macrophages using Anova because the research data is parametric and exceeds 2 variables and the results were obtained ($P < 0.05$) which showed differences in the three groups (Table 1).

The post-hoc test was conducted find significance difference between groups. Since the results of PMN and macrophages tests were normal but not homogenous, the Games Howell post-hoc test (Table 2) were used to find out which groups have significant differences between the control group and the exercise group.

The results of the post-hoc test showed significant differences in all groups. This shows that there were significant differences in the number of PMNs and macrophages after tooth extraction in wistar rats given aerobic and anaerobic exercise.

4. Discussion

The results of this study indicate differences in the average number of PMN cells and macrophages between the control group and the treatment group. The results of PMN calculations in the control group had an average number of cells of 22.286, in aerobic exercise group the average number of PMN cells was 44.6000, and in anaerobic exercise group the average number of PMN cells was 35.2000. The results of macrophage cell count in the control group obtained an average of 16.8571, in the aerobic exercise group obtained an average of 42.5714, and in anaerobic exercise group the average was 29.6571. On the results of the different tests between groups using the post-hoc test Gowes Well type showed a significant difference in all groups, between the control group and the exercise group (Fig. 1).

The process of healing wounds after tooth extraction, shortly after an injury occurs inflammatory cells such as PMN and macrophages become very active and require a lot of oxygen for the metabolic process to be able to phagocytes microorganisms and necrotic tissue. This is what makes the condition in the area around the wound become hypoxia, the condition when the oxygen concentration in cells is very low. Hypoxia conditions in acute wounds can activate HIF-1, this will initiate the process of expression of hypoxia responsive

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Table 2 Post-hoc games Howell Test.

Post-hoc games Howell	K (control)	P1 (aerobic)	P2 (anaerobic)
PMN cell			
P1 (aerobic)	22.37143[a]	–	–
P2 (anaerobic)	12.97143[a]	−9.40000[a]	–
Macrophage cell			
P1 (aerobic)	25.71429[a]	–	–
P2 (anaerobic)	12.80000[a]	−12.91429[a]	–

[a] The results significance if (*P*-value < 0/*P*-value > 1).

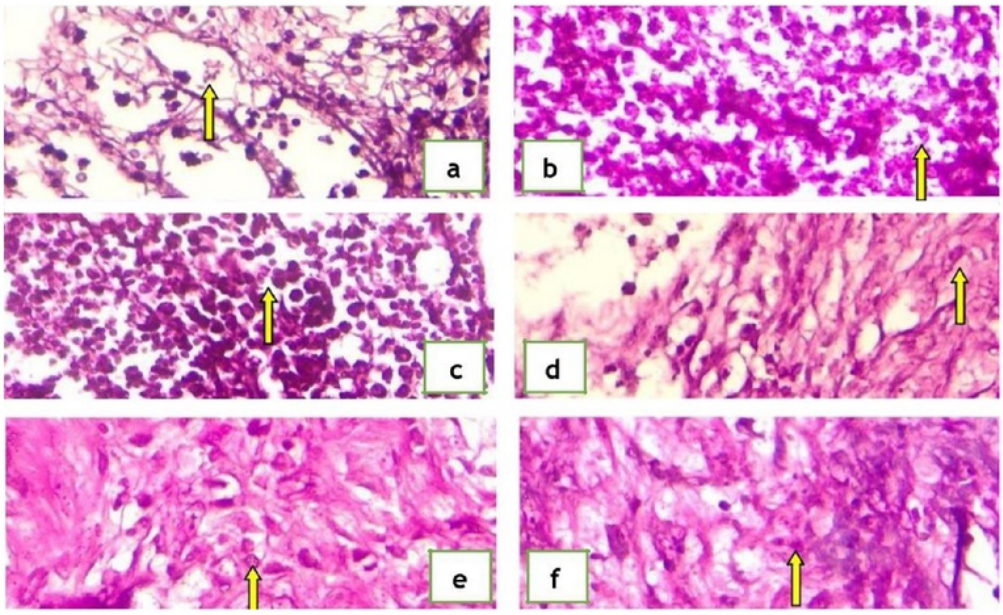

Figure 1 PMN cells (a) and macrophage cells (b) on control group (K); PMN cells (c) and macrophage cells (d) on aerobic exercise group (P1); PMN cells (e) and macrophage cells (f) on anaerobic exercise group (P2) with HE (Hematoxylin Eosin), 400 × .

genes. However, this condition should not be allowed for a long time because the wound healing process in the wound still requires a sufficient amount of tissue oxygenation. In chronic hypoxia conditions will have a negative impact on the human body. Therefore, it needed sufficient oxygen supply to prevent damage on the wound area and healing process could run properly [15,16]. PMN and macrophages in the inflammatory phase have the function of phagocytosis to digest and destroy bacteria, producing toxic content in the form of lactoferin, proteases, neutrophil elastase, and catepsin which can destroy bacteria, and increase free radicals (ROS) and when ROS production become excessive the wound healing process may be disrupted. Activated macrophage infiltration increases nitric oxide concentration (NO) which is a diatomic free radical produced by activated phagocytic leukocytes (neutrophils and macrophages) where both have dangerous properties and also beneficial in tissue pathophysiological process [4,17]. PMN also release FGF, TGF-β, TGF-α, and PDGF which will help fibroblasts proliferation and angiogenesis in the next phase of healing process. Macrophages can also modify their metabolic function for healing or growth promoting setting (M2 macrophages) by synthesizing growth factor such as TGF, cytokines and IL-1, TNF, FGF, PDGF, EGF, dan VEGF [18–20].

In PMN and macrophage with aerobic treatment groups have the highest average number of cells because in aerobic exercise can increase VO_{2max}, which is the maximum amount of oxygen that could be consumed during physical exercise. This VO_{2max} increase cause more amount of oxygen in the tissue and help tissue that suffered hypoxia. Adequate oxygen is needed to prevent negative effects on human body, minimalize damage in wound area so the healing process could proceed properly. This affects the activation and work of PMN and macrophages that need lots of oxygen for their metabolic processes to be able to phagocytes microorganisms and necrotic tissue. Tissue conditions that contain a lot of oxygen will cause the work of PMN cells and

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macrophage cells more maximal, effective and faster. So that the healing process in the following phases will also be faster [21].

In PMN cells and macrophage cells, anaerobic exercise has an average number of cells less than the control group because each anaerobic physical exercise could lead to the emergence of free radicals (ROS) in which if the human body could not compensate, it will become oxidative stress and could damage cell components to maintain cell integrity. In excessive weight training, trauma will occur then continue to inflamed. Inflammation process will activate phagocytes cells, especially PMN to migrate to the injured area. PMN will phagocytes foreign substances, and release proteolytic enzyme to do degranulation, and the last process will form free radicals to break foreign substances. This enzyme is released by Nicotinamide Adenine Dinucleotide (NADPH) oxidase. This enzyme trigger the formation of free radicals. In the healing process itself produce free radical and are added with free radicals produced from anaerobic physical exercise. This can interfere with the wound healing process because of the imbalance between free radical production and the human body's antioxidant defense system that cause cell damage and interfere cell function so it will interrupt wound healing process [22,23].

In PMN cells and macrophage cells, aerobic and anaerobic exercise has an average number of cells more than the control group because each physical exercise that pays attention to the principle of physical exercise (intensity, frequency, duration, and type) plays a role in physical fitness and survival of an organism and impacts on optimal physiological performance of the body.

In all groups, control, aerobic and anaerobic exercise showed that there were significant differences in the number of both PMN and macrophage cells. The results of this study are also suitable with previous studies that prove that the process of healing post-extraction wounds can be improved by doing aerobics physical exercise, but in this study using different exercise principles [24].

5. Conclusions

This research is succeeded in showing significant differences in the number of PMN and macrophage cells as an indicator of the tooth extraction wound healing acceleration in inflammatory phase in groups without exercise, aerobic exercise, anaerobic exercise. In the physical exercise group is better than group without physical exercise, while in aerobic exercise group is better than anaerobic exercise group.

Disclosure of interest

The authors declare that they have no competing interest.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6